

Appl. No. 09/913,467  
Amdt. dated April 5, 2006  
Reply to Office Action of January 5, 2006

PATENT

**Amendments to the Claims:**

This listing of claims will replace all prior versions, and listings of claims in the application:

**Listing of Claims:**

1. (Currently Amended) A bone morphogenetic protein (BMP) or a growth differentiation factor (GDF) polypeptide variant with increased heparin-binding ability, characterized in that
- (i) added to the amino acid sequence of a BMP or GDF polypeptide is at least one oligopeptide comprising the amino acid sequence  $X_1X_2X_3X_4X_5X_6$ ; and/or
  - (ii) inserted into the amino acid sequence of a BMP or GDF polypeptide is at least one oligopeptide comprising the amino acid sequence  $X_1X_2X_3X_4X_5X_6$ ; and/or
  - (iii) at least one oligopeptide sequence naturally occurring within the amino acid sequence of a BMP or GDF polypeptide is substituted by an oligopeptide comprising an amino acid sequence  $X_1X_2X_3X_4X_5X_6$ ,

wherein

$X_1 = K, R, \text{ or } H$ ;

$X_2 = K, R, \text{ or } H$ ;

$X_3 = K, R, \text{ or } H, \text{ or no amino acid}$ ;

$X_4 = \text{not } K, R, H, \text{ but any other amino acid}$ ;

$X_5 = \text{not } K, R, H, \text{ but any other or no amino acid}$ ;

$X_6 = \text{not } K, R, H, \text{ but any other or no amino acid (SEQ ID NO: 1)}$ , and

~~the polypeptide is a bone morphogenetic protein (BMP) or a growth differentiation factor (GDF).~~

2. (Currently Amended) A BMP or GDF polypeptide variant as recited in claim 1, characterized in that one to four copies of said oligopeptide are inserted at one to four positions within the BMP or GDF polypeptide.

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3. (Withdrawn - Currently Amended) A bone morphogenetic protein (BMP) or a growth differentiation factor (GDF) polypeptide variant as recited in claim 1, characterized in said oligopeptide comprises amino acid sequence RKRA (SEQ ID NO:3) or RKRAKHKQ (SEQ ID NO:4) with increased heparin-binding ability, characterized in that

- (i) added to the amino acid sequence of a polypeptide is at least one oligopeptide comprising the amino acid sequence RKRA (SEQ ID NO:3) or RKRAKHKQ (SEQ ID NO:4); and/or
- (ii) inserted into the amino acid sequence of a polypeptide is at least one oligopeptide comprising the amino acid sequence RKRA (SEQ ID NO:3) or RKRAKHKQ (SEQ ID NO:4); and/or
- (iii) at least one oligopeptide sequence naturally occurring within the amino acid sequence of a polypeptide is substituted by an oligopeptide comprising an amino acid sequence RKRA (SEQ ID NO:3) or RKRAKHKQ (SEQ ID NO:4).

4. (Currently Amended) A BMP or GDF polypeptide variant as recited in claim 1, characterized in that said oligopeptide is added to the N-terminus and/or inserted into the N-terminal region, and/or substitutes a part of the N-terminal region.

5. (Currently Amended) A BMP or GDF polypeptide variant as recited in claim 1, characterized in that the amino acid sequence of said BMP or GDF polypeptide variant further contains a sequence of relevance to recombinant expression at the N-terminus, said sequence of relevance to recombinant expression being M or MZ, where M stands for methionine and Z stands for one or more amino acids.

6. (Currently Amended) A BMP or GDF polypeptide variant as recited in claim 1, characterized in that said BMP or GDF polypeptide variant further contains a His-tag.

7. (Currently Amended) A BMP or GDF polypeptide variant as recited in claim 1, characterized in that said BMP or GDF polypeptide is altered by addition, substitution,

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insertion, inversion, and/or deletion, where said BMP or GDF polypeptide altered by addition, substitution, insertion, inversion and/or deletion shows at least 10% of the biological activity of the unaltered polypeptide essentially the same receptor binding affinity to the ectodomain of BMPR-IA as BMP-2, and at least 90% homology to the unaltered BMP or GDF polypeptide.

8. (Currently Amended) A BMP or GDF polypeptide variant as recited in claim 1, characterized in that said BMP or GDF polypeptide is BMP-2, BMP-4, BMP-5, BMP-6, BMP-7/OP-1, BMP-8/OP-2, or GDF5.

9. (Currently Amended) A BMP or GDF polypeptide variant as recited in claim 1, wherein the BMP or GDF polypeptide has a cysteine knot, characterized in that said oligopeptide is inserted before the cysteine knot.

10. (Withdrawn - Currently Amended) A bone morphogenetic protein 2 (BMP-2) polypeptide variant as recited in claim 8 with increased heparin-binding ability, characterized in that said BMP-2 polypeptide variant has the amino acid sequence SEQ ID NO:5 (T3) or SEQ ID NO:6 (T4).

11. (Currently Amended) A BMP or GDF polypeptide variant as recited in claim 1, characterized in that said BMP or GDF polypeptide variant is a polymer, oligomer, or dimer.

12. (Currently Amended) A nucleic acid molecule, comprising a nucleic acid sequence encoding a BMP or GDF polypeptide variant as recited in claim 1.

13. (Original) A nucleic acid molecule as recited in claim 12, characterized in that said nucleic acid sequence is derived from genomic DNA or cDNA, or is a synthetic DNA.

14. (Currently Amended) A nucleic acid molecule as recited in claim 12, further comprising a promoter suited to control expression, wherein said nucleic acid sequence encoding a BMP or GDF polypeptide variant is under the control of said promoter.

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15. (Previously Presented) A nucleic acid molecule as recited in claim 12, wherein said nucleic acid molecule contains at least part of a vector.

16. (Previously Presented) An isolated host cell, containing a nucleic acid molecule as recited in claim 12, wherein said host cell is a prokaryotic or eukaryotic cell suitable for expression of said nucleic acid molecule.

17. (Currently Amended) A process for producing a BMP or GDF polypeptide variant with increased heparin-binding ability as recited in claim 1, comprising:  
addition to the amino acid sequence of a BMP or GDF polypeptide of at least one oligopeptide containing an amino acid sequence selected from SEQ ID NO:1; and/or  
insertion into the amino acid sequence of a BMP or GDF polypeptide of at least one oligopeptide containing an amino acid sequence selected from SEQ ID NO:1; and/or  
substitution of at least one oligopeptide sequence naturally occurring within the amino acid sequence of a BMP or GDF polypeptide by one oligopeptide containing an amino acid sequence selected from SEQ ID NO:1.

18. (Original) A process as recited in claim 17, characterized in that said process comprises a chemical and/or enzymatic synthesis process.

19. (Previously Presented) A process as recited in claim 17, characterized in that said process comprises gene technological processes.

20. (Currently Amended) A process as recited in claim 17, characterized in that said process comprises:

a) in vitro mutagenesis of a nucleic acid encoding a BMP or GDF polypeptide, so that

(i) to the nucleic acid encoding said BMP or GDF polypeptide is added at least one nucleic acid encoding an oligopeptide containing an amino acid sequence that is selected from SEQ ID NO:1; and/or

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(ii) into the nucleic acid encoding said BMP or GDF polypeptide is inserted at least one nucleic acid encoding an oligopeptide containing an amino acid sequence that is selected from SEQ ID NO:1; and/or

(iii) at least one nucleic acid sequence naturally occurring within the nucleic acid sequence encoding said BMP or GDF polypeptide is substituted by a nucleic acid sequence encoding an oligopeptide containing an amino acid sequence selected from SEQ ID NO:1;

b) cloning of the mutated nucleic acid into a suitable expression vector;  
c) transformation/transfection of a suitable host cell with the expression vector obtained;

d) cultivation of said transformed/transfected host cell under conditions suitable for expression;

e) isolation, and if necessary renaturation, of the expressed polypeptide variant.

21. (Previously Presented) A process as recited in claim 17, characterized in that said process is carried out within a prokaryotic host cell.

22. (Previously Presented) A process as recited in claim 17, characterized in that said process is carried out within a eukaryotic cell.

23. (Currently Amended) A pharmaceutical composition, comprising a BMP or GDF polypeptide variant as recited in claim 1.

24. (Canceled)

25. (Currently Amended) A composition, comprising a BMP or GDF polypeptide variant as recited in claim 1 and a carrier selected from among heparin, hydroxyapatite, hyaluronic acid, synthetic polymers, and collagen.

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26. (Currently Amended) A matrix, characterized in that said matrix contains or is coated with heparin or heparin-like substances and BMP or GDF polypeptide variants as recited in claim 1 are adsorbed to said heparin or heparin-like substances.

27. (Previously Presented) A process as recited in claim 21, wherein the prokaryotic host cell is *E. coli*.

28. (Previously Presented) A process as recited in claim 22, wherein the eukaryotic cell is selected from the group consisting of a yeast cell, a plant cell, an insect cell, CHO cells, and COS cells.

29. (Canceled)